Abstract No. von562

A Powder Diffraction Study of the Binding of N-acetylglucosamine to Chicken Egg Lysozyme

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Beamline(s): X3B1

Introduction: The experimental verification of the binding mode for small molecule ligands to proteins under a wide variety of conditions is needed to understand the mechanisms of protein action and inhibition. This work is an attempt to examine the interaction between N-acetylglucosamine and lysozyme by high-resolution x-ray powder diffraction..

Methods and Materials: Powder diffraction samples were prepared by combining 25mg chicken egg lysozyme with 10mg N-acetylglucosamine (NAG) in 200μl pH 5.0 and 6.0 0.5M NaCl buffers in an agate mortar. The resulting slurry for each case was loaded into a 1.5mm diameter glass capillary, centrifuged and the sealed to prevent evaporation. Powder patterns were collected from 1-14^{o} 2Θ at λ=0.70A in 0.002^{o} steps over 10-12h on line X3B1. A comparison of the powder diffraction patterns of the materials with and without NAG showed a clear indication of complex formation from the pH 6.0 buffer but not the pH 5.0 one (Fig. 1a&b). Analysis of this diffraction data was done with GSAS and ΔF maps generated from extracted structure factors generated during preliminary Rietveld refinements showed that NAG only bound at pH 6.0.

Results: Analysis of the high-resolution powder diffraction data from NAG/lysozyme complex formed in pH 6.0 0.5M NaCl buffer shows that the NAG ligand is bound to the C-binding site on lysozyme (Fig 2).

Conclusions: Clearly high-resolution x-ray powder diffraction can be used to investigate protein/ligand complexes and useful structural results can be obtained from this technique.

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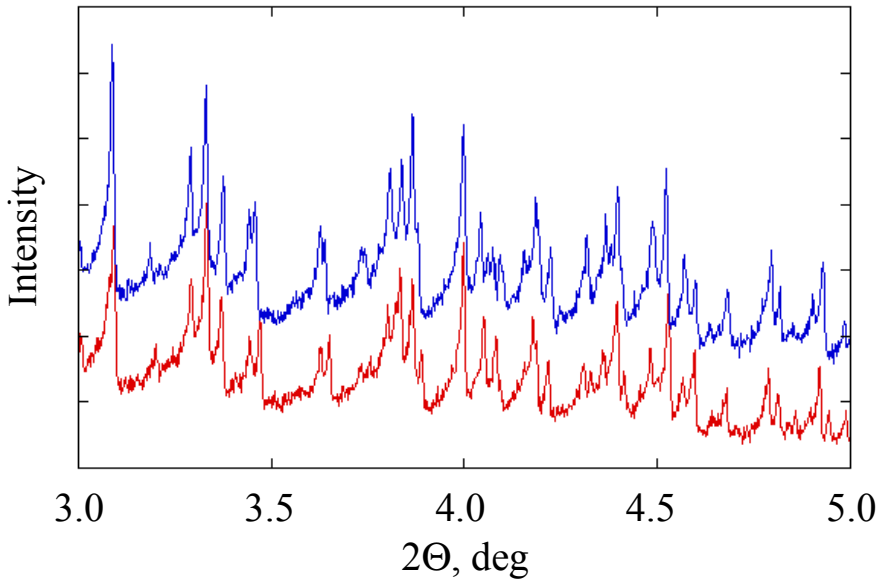

Figure 1a. A small segment of high resolution x-ray powder diffraction patterns of lysozyme (red) and lysozyme/N-acetylglucosamine mixture (blue) precipated from pH6.0 0.5 M NaCl buffer taken with λ=0.70Å. The latter pattern has been offset for clarity.

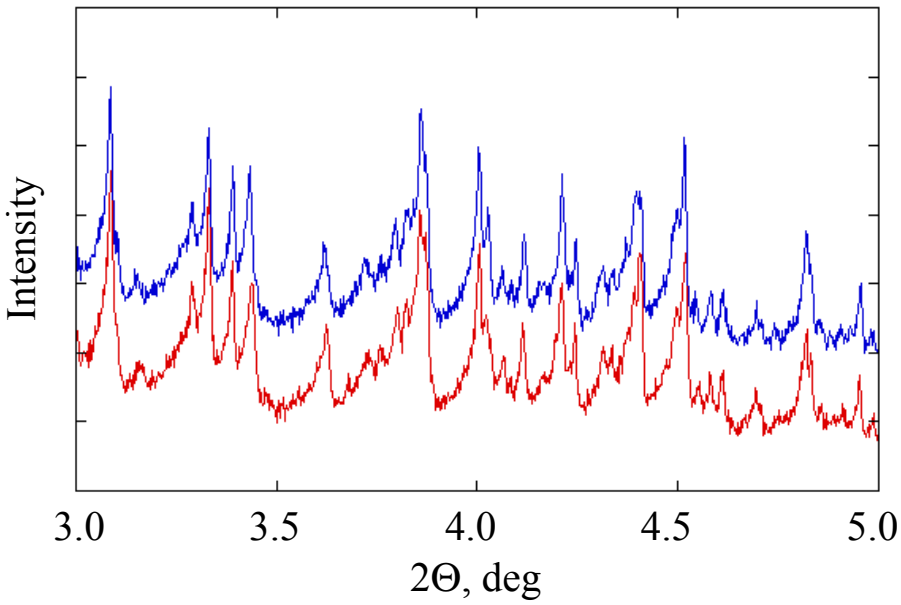

Figure 1b. A small segment of high resolution x-ray powder diffraction patterns of lysozyme (red) and lysozyme/N-acetylglucosamine mixture (blue) precipated from pH 5.0 0.5 M NaCl buffer taken with λ=0.70Å. The latter pattern has been offset for clarity.

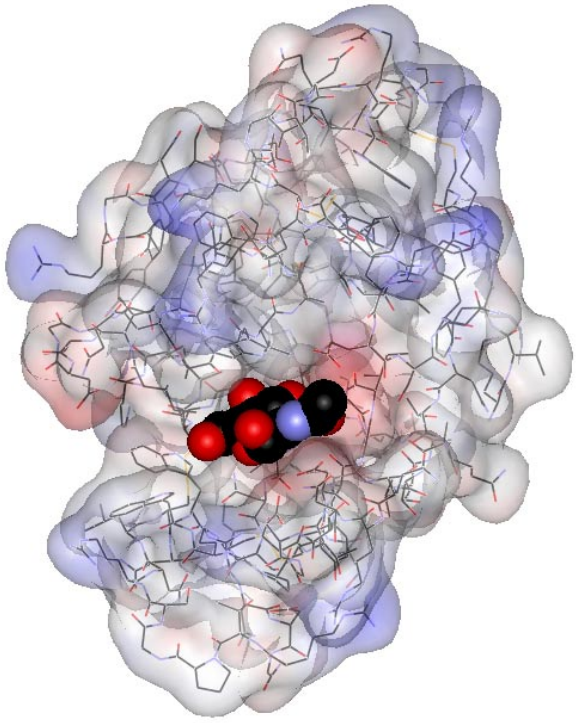

Figure 2. Structure of the NAG/lysozyme complex as determined from high-resolution x-ray powder diffraction. The NAG ligand is in the C binding site of lysozyme.